

Redoing A0, A2 and AI2

	PCR 20 µL reaction (units : µL)	Final Concentration				
10x thermopol reaction buffer	2,5	1X				
10 mM dNTPs	0,5	200 µM				
10 µM Forward Primer	0,5	0.2 µM				
10 µM Reverse Primer	0,5	0.2 µM		Primers:	r_Squ_ADH1gRNA	
Template (colony)	0	5 ng			f_Squ_ADH1gRNA	
DMSO (optional)	0	3 %				
Taq polymerase	0,125	1.0 units/50 µl PCR				
Nuclease-free Water	20,875					
Total volume	25					
Number of Reactions	33	10x3 samples + 1 negative control +2 extra		A0-11	A2-11	AI2-11
				A0-12	A2-12	AI2-12
10X Reaction buffer	82,5			A0-13	A2-13	AI2-13
dNTPs	16,5			A0-14	A2-14	AI2-14
r_Squ_ADH1gRNA	16,5			A0-15	A2-15	AI2-15
f_Squ_ADH1gRNA	16,5			A0-16	A2-16	AI2-16
Taq Polymerase	4,125			A0-17	A2-17	AI2-17
Water	688,875			A0-18	A2-18	AI2-18
Total volume	825			A0-19	A2-19	AI2-19
Volume per tube	25			A0-20	A2-20	AI2-20
PCR program A 'Y-COL-D'						
Initial Denaturation	95°	7 min				
	95°	30 sec				
	52°	1 min				
35 Cycles	67°	1 min 20				
Final Extension	72°	5 min				
Hold	4°					